Bacteriophages as a model for studying carbon regulation in aquatic system

Swapnil G Sanmukh[1], Waman N Paunikar*[1], Sandhya Swaminathan[2] and Satish K Lokhande[1]

[1*]Corresponding Author: Ecosystem Division, National Environmental Engineering Research Institute (NEERI), Nagpur-440020, Maharashtra, India. Telephone: 0712-2249885-9, Fax: 0712-2249762, E-mail: wn_paunikar@neeri.res.in.

[2]NEERI Zonal laboratory, CSIR Complex, T.T.T.I., Taramani P.O. Chennai – 600113, Tamil Nadu, India Phone: 044-22541250, 044-22541964, Fax: 044-22541964, E-mail: s_sandhya@neeri.res.in

ABSTRACT:

The interconversion of carbon in organic, inorganic and refractory carbon is still beyond the grasp of present environmentalists. The bacteria and their phages being the most abundant constituents of the aquatic environment, represents an ideal model for studing carbon regulation in aquatic system. The refractory dissolved organic carbon (DOC) a recently coined terminology from the microbe-driven conversion of bioavailable organic carbon into difficult-to-digest refractory DOC by microbial carbon pump (MCP) is suggested to have potential to revolutionize our view of carbon sequestration. It is estimated that about 95% of organic carbon is in the form of refractory DOC which is the largest pool of organic matter in the ocean. The refractory DOC is supposed to be the major factor in the global carbon cycle whose source is not yet well understood. A key element of the carbon cycle is the microbial conversion of dissolved organic carbon into inedible forms. The time studies of phage-host interaction under control conditions reveals their impact on the total carbon content of the source and their interconversion among organic, inorganic and other forms of carbon with respect to control source. The TOC- analysis statistics stipulate increase in inorganic carbon content by 15-25 percent in the sample with phage as compared to sample without phage. The results signify 60-70 fold increase in inorganic carbon content in sample with phage, whereas, 50-55 fold in the case of sample without phages as compared with control. This increase in inorganic carbon content may be due to lysis of the host cell releasing its cellular constituents and utilization of carbon constituent for phage assembly and development. It also proves

the role of phages in regulating the carbon flow in the aquatic systems like oceans where their concentration outnumbered other species.

KEYWORDS: interconversion, refractory carbon, microbial carbon pump, carbon sequestration, global carbon cycle.

INTRODUCTION:

The regulation of carbon in aquatic system is one of the major processes among biogeochemical cycles. The inedible or refractory dissolved organic carbon is a recently coined terminology from the microbe-driven conversion of bioavailable organic carbon into difficult-to-digest refractory DOC by microbial carbon pump (MCP). This concept is suggested by some workers that have potential to revolutionize our view of carbon sequestration. It is also said that the ocean surface take up about 2% more CO_2 gas than they release of which some amount of CO_2 dissolves into the water, forming carbonic acid. The increase in level of CO_2 in oceans decreases the pH resulting in acidification which affects the aquatic ecosystem. Carbon also enters the seas through the food web through photosynthesis, but does not lasts for long period and is released to the atmosphere as CO_2, whereas, some in the form of remains of dead organic matters sink at the ocean depth. However, much more amounts of carbon are in the water as DOC. It is estimated by some workers that about 95% of organic carbon is in the form of refractory DOC which is the largest pool of organic matter in the ocean. The refractory DOC is supposed to be the major factor in the global carbon cycle whose source is not yet well understood [2], [4] and [5].

Viruses are by far the most abundant 'lifeforms' in the oceans and are the reservoir of most of the genetic diversity in the sea. The estimated 10^{30} viruses in the ocean and every second, approximately 10^{23} viral infections occur in the ocean. These infections are a major source of mortality, and cause disease in a range of organisms, from shrimp to whales. Hence, viruses influence the composition of marine communities and are a major force behind biogeochemical cycles [1].

A key element of the carbon cycle is the microbial conversion of dissolved organic carbon into inedible forms. Microbes play a dominant role in “pumping” bioavailable carbon into a pool of relatively inert compounds. The MCP “may act as one of the conveyor belts that transport and store

carbon in the deep oceans." The MCP also appears to function in deep waters, where bacteria adapted to the high-pressure environment may have "a special capacity" to degrade refractory DOC. In a landmark paper in 2001, Hiroshi Ogawa et al., showed that marine microbes are able to convert bioavailable DOC to refractory DOC [2], [4] and [5].

The present communication represents the time studies of phage-host interaction under control conditions, to analyze their impact on the total carbon content of the source (Nutrient broth) and their interconversion among organic, inorganic and other forms of carbon with respect to control. The data generated is based on the results obtained from TOC-analyzer (Figure 3 & 4).

MATERIALS AND METHODS:

We used sterilized Nutrient broth media for inoculation of E. coli (ATCC 13706) strain and incubate it at 37^0 C for 18 hours in 4 conical flasks. The 2 flasks of control broth were not inoculated with bacterium and were preserved in refrigerator at 4^0 C after autoclaving till the experiment begins. The initial reading of all 6 cultures were analysed by TOC (Total Organic Carbon) analyzer after 18 hours of incubation. The phage phi X174 ATCC 13706 B1 were added to the 2 conical flasks containing E.coli (ATCC 13706) strain after taking samples for initial reading. The analysis was further carried out after every 2 hours till the stationary state is achieved in the results. The experiment was carried out in duplicates so as to avoid the effect of time factor and manual error on the results obtained.

The experiment was designed to measure the inorganic carbon from three sets viz.

a) Control sample,
b) Sample with bacteria and
c) Sample with bacteria and its specific phage

The bacterium used during our study was E. coli (ATCC 13706) and the bacteriophage used was phi X174 ATCC 13706 B1. The nutrient broth was used for all three set of experiment [3]. The study the effect of phage–host interaction on the carbon regulation we prepared three sets of samples namely;

a. Control sample

The control sample prepared was of nutrient broth [3] devoid of any bacterial or viral inoculation and was stored at 4^0C throughout the experimentation process. It was

kept in duplicate so as to minimize the manual or instrumental error if any.

b. Sample with bacteria

The Sample with host (Bacteria) was prepared in nutrient broth [3] which was inoculated with E.coli strain ATCC-13706 and was incubated at 37^0C throughout the experimentation process in duplicates.

c. Sample with bacteria and its specific phage

The Sample with host (Bacteria) and phage was prepared in nutrient broth [3] which was inoculated with E.coli strain ATCC-13706 and its specific phage phi X174 ATCC 13706 B1 which was incubated at 37^0C throughout the experimentation process in duplicates.

RESULTS AND DISCUSSION:

The results of the three sets are represented in Table 1 & 2, which clearly show that the inorganic carbon content of the samples is increased with respect to time (except control) in all the two sets. The sample set with host-phage inoculation show greater reading of inorganic carbon with respect to the sample with host alone. There is an average 20-25 ppm increase in inorganic carbon composition of sample set with host-phage inoculation. The result indicates that the phages may be advantageous in regulation of carbon in aquatic systems by carbon sequestration.

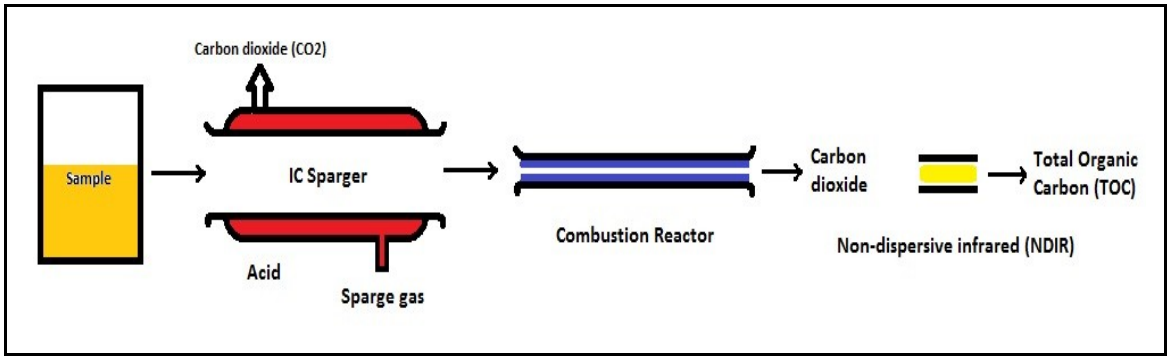

FIGURE 1. PRINCIPLE OF TOC ANALYSIS

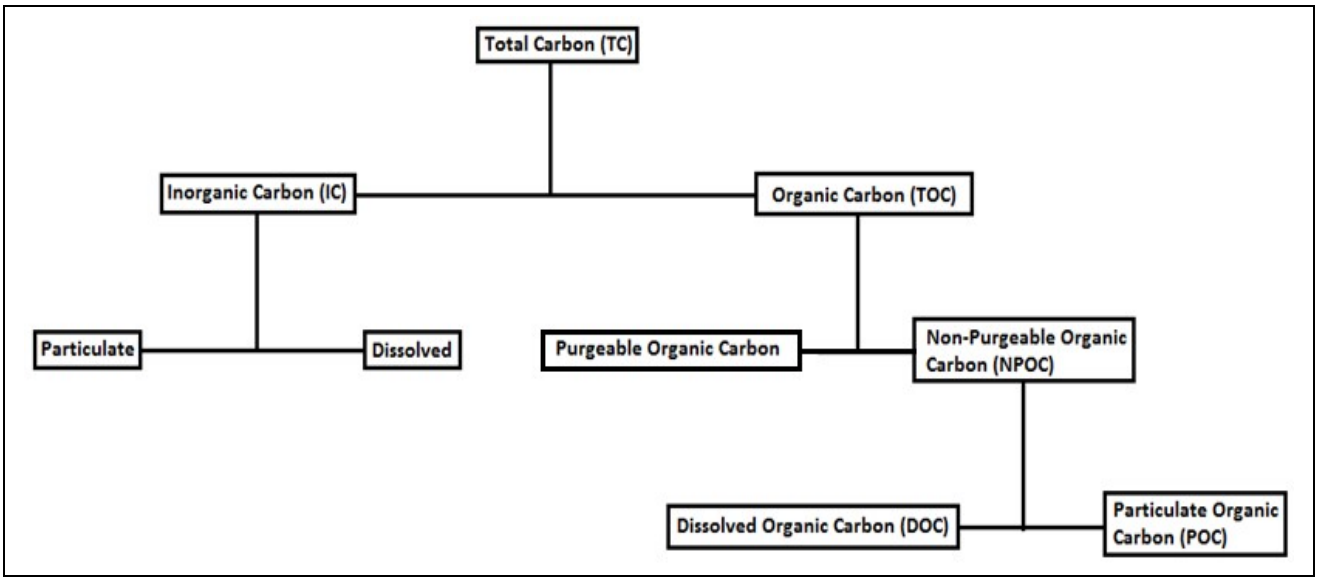

FIGURE 2. FLOW CHART SHOWING INGREDIENT COMPONENTS OF TOTAL CARBON

TABLE 1. TOC ANALYSIS RESULTS OF CONTROL AND BACTERIAL SAMPLES (WITH AND WITHOUT PHAGE)

Experiment No. 1	Control 1 (ppm)			Sample without phage 1 (ppm)			Sample with phage 1 (ppm)		
Time (hours)	TOC	TC	IC	TOC	TC	IC	TOC	TC	IC
0	2915	2916	0.7118	2740	2769	28.91	2780	2811	31.53
2	2834	2834	0.9182	2818	2847	28.91	2788	2818	29.72
4	2507	2508	0.9432	2162	2193	29.86	2209	2239	31.38
6	2436	2437	0.8439	2301	2327	24.77	2517	2543	25.34
8	2152	2153	1.064	1921	1946	22.27	1906	1929	25.89
10	1929	1930	0.8917	1530	1562	22.24	1372	1394	31.51
12	1887	1888	0.9637	1757	1798	31.27	1496	1528	31.93
14	1827	1828	0.9217	1415	1458	43.09	1759	1809	50.66
24	2880	2882	1.238	2689	2784	94.76	2648	2764	116.4
26	2741	2742	1.751	2726	2811	85.83	2684	2789	105.5
28	3332	3333	1.557	3047	3126	79.59	3091	3196	105.5

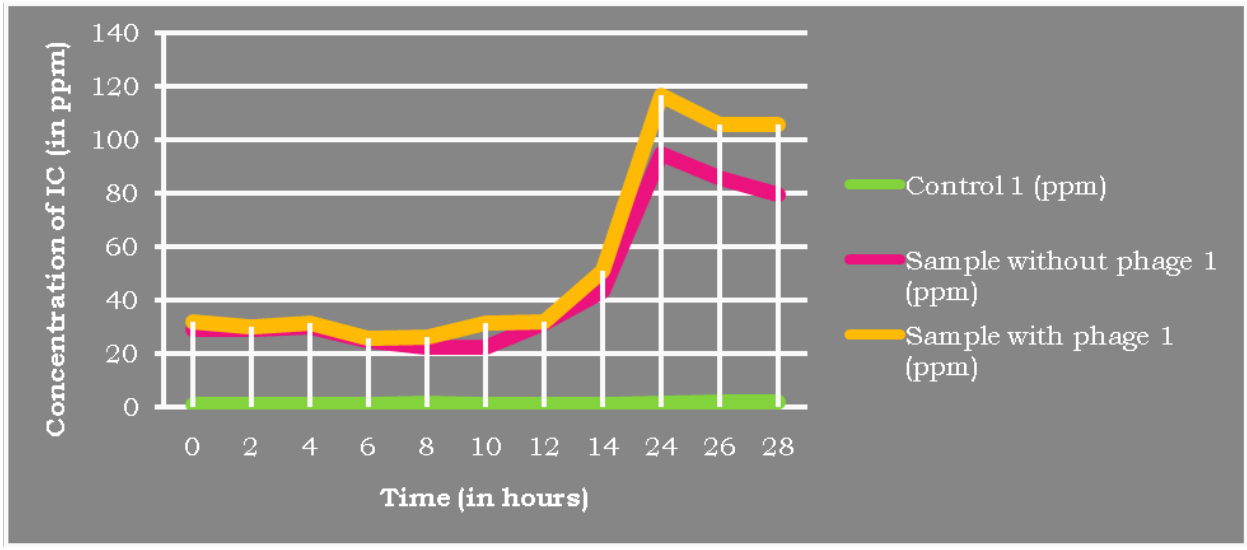

FIGURE 3. VARIATION IN INORGANIC CARBON CONTENT (IN PPM) WITH RESPECT TO TIME (IN HOURS)

TABLE 2. TOC ANALYSIS RESULTS OF CONTROL AND BACTERIAL SAMPLES (WITH AND WITHOUT PHAGE)

Experiment No. 2	Control 2 (ppm)			Sample without phage 2 (ppm)			Sample with phage 2 (ppm)		
Time (hours)	TOC	TC	IC	TOC	TC	IC	TOC	TC	IC
0	3041	3042	0.7992	2789	2818	28.96	2844	2871	27.47
2	2871	2872	0.9459	2922	2951	28.61	2756	2794	37.72
4	2573	2574	0.8808	2360	2389	29.13	2365	2396	31.26
6	2167	2168	0.8449	2345	2370	24.77	2286	2319	33.11
8	2184	2185	1.039	1935	1957	23.16	1953	1983	30.04
10	1456	1457	1.004	1574	1600	25.94	1536	1570	33.44
12	1907	1908	0.9637	1819	1852	34.15	1592	1630	37.37
14	1631	1632	0.9014	2032	2115	64.52	2023	2088	82.56
24	2679	2681	1.421	2752	2853	100.9	2538	2657	119
26	2773	2775	1.533	2779	2877	98.77	2701	2818	116.8
28	3244	3245	1.65	3157	3250	92.22	3005	3113	107.2

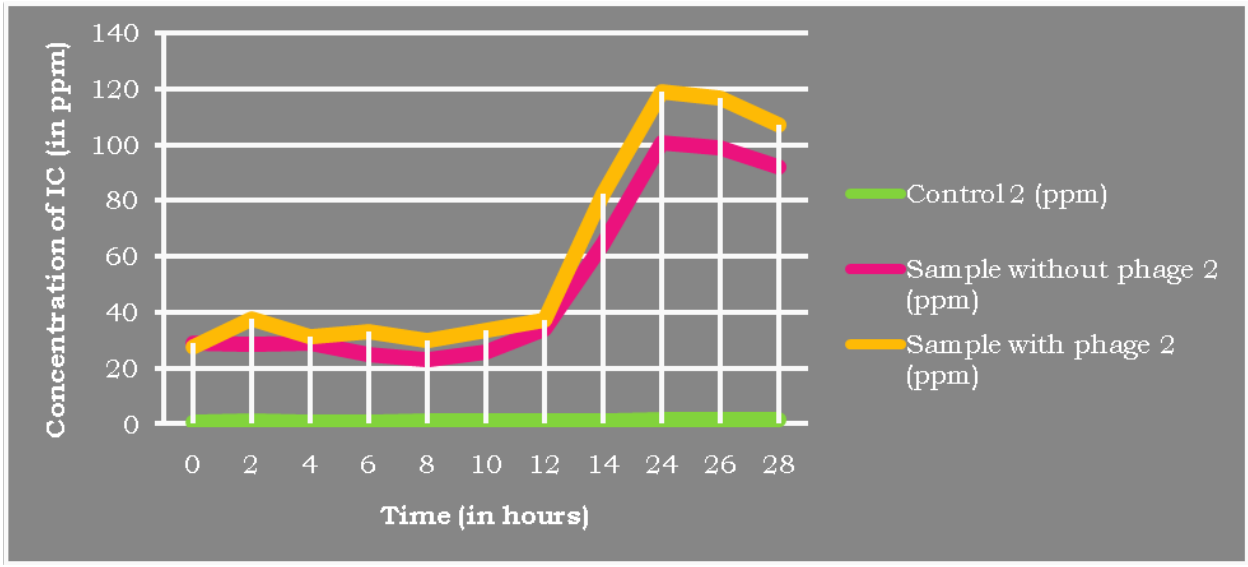

FIGURE 4. VARIATIONS IN INORGANIC CARBON CONTENT (IN PPM) WITH RESPECT TO TIME (IN HOURS)

CONCLUSION:

The increase in inorganic carbon content may be due to lysis of the host cell releasing its cellular constituents and utilization of carbon constituent for phage assembly and development. It also proves the role of phages in regulating the carbon flow in the aquatic systems like oceans where their concentration outnumbered other species.

ACKNOWLEDGMENT:

We would like to thank Mr. Dilip Budha Meshram, Research fellow, AAE Division, NEERI, Nagpur and Mr. Masoodur Rahman, trainee, AAE Division, NEERI, Nagpur for providing practical help in carrying out this experiment successfully.

REFERENCES:

[1] C.A. Suttle. Marine viruses- major players in the global ecosystem. Nature Reviews Microbiology 5, 2007, 801-812

[2] H. Ogawa, Y. Amagai, I. Koike, K. Kaiser and R. Benner. "*Production of Refractory Dissolved Organic Matter by Bacteria*." Science. 2001, 292, 917-920.

[3] L. S. Clescerl, A. E. Greenberg and A. D. Eaton (1999). "*Standard Methods for Examination of Water & Wastewater (20th ed.).*" Washington, DC: American Public Health Association.

[4] R. Stone. "*The invisible hand behind a vast carbon reservoir.*" Science. 2010, 328, 1476.

[5] S. K. Zbigniew, F. G. Plumley, A. S. Lang, J. T. Beatty, R. E. Blankenship, C. L. VanDover, C. Vetriani, M. Koblizek, C. Rathgeber and P. G. Falkowski. "*Contribution of aerobic photoheterotrophic bacteria to the carbon cycle in the ocean.*" Science. 2001, 292, 2492.

Mr. Swapnil Ganesh Sanmukh, born on 07th November 1985 in Miraj, Dist- Sangli, Maharashtra, India. He had completed his

Masters in Environmental Biotechnology from Shivaji University, Kolhapur, Maharashtra in 2009. Now, pursuing his Ph.D in Environmental Biotechnology from University of Madras, Chennai-600113, Tamil Nadu, India under the guidance of Dr. S. Swaminathan, is working as a Deputy Director & Head, National Environmental Engineering Research Institute (NEERI), Chennai zonal laboratory, Tamil Nadu-600113, India (e-mail: s_sandhya@neeri.res.in). His major fields of interest are Microbiology, Molecular biology and Bioinformatics. He is now working as a Ph.D. Research fellow in Applied Aquatic Ecosystem Division, National Environmental Engineering Research Institute, Nagpur-440020, Maharashtra, India.(e-mail: swamukh1985in@rediffmail.com).

Dr. Waman Narayan Paunikar, born on 9th October 1955 in Mohadi, Dist-Bhandara, Maharashtra, India. He had completed his Masters in Zoology (Cell biology) from Nagpur University, Nagpur, Maharashtra in 1980. He was Ph.D. in Zoology from R.T.M. Nagpur University, Nagpur, Maharashtra in 2011. His major fields of interest are Virology, Bacteriology, Molecular biology and Bioinformatics. He is now working as Principal scientist in Applied Aquatic Ecosystem Division, National Environmental Engineering Research Institute, Nagpur-440020, Maharashtra, India. Dr. W. N. Paunikar is working as a Principal Scientist in Applied Aquatic Ecosystem Division, National Environmental Engineering Research Institute (NEERI), Nagpur-440020, Maharashtra, India (phone: +91-9823822766, fax: +91-712-2249900, e-mail: wn_paunikar@neeri.res.in).

Dr. S. Swaminathan, is working as a Deputy Director & Head, National Environmental Engineering Research Institute (NEERI), Chennai zonal laboratory, Tamil Nadu-600113, India (e-mail: s_sandhya@neeri.res.in).

Er. S. K. Lokhande, is working as a Technical Assistant in Analytical Instrumentation Division, National Environmental Engineering Research Institute (NEERI), Nagpur-440020, Maharashtra, India (e-mail: s_lokhande@neeri.res.in).